

## ***In situ* Quantification of the Impact of Episodic Enhanced Turbulent Events on Large Phytoplankton**

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### **LONG-TERM GOALS**

Our long-term goal is to understand how physical-biological, biological-biological and chemical-biological interactions control the patch structure and ecology of phytoplankton inhabiting coastal shelves, upwelling areas, fjords and banks. We are especially interested in ways in which species-specific properties, including colony size and shape (diatoms) and motility (flagellates and photosynthetic ciliates) interact with physical processes to regulate phytoplankton dynamics and spatial-temporal distribution patterns. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, biodiversity, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

### **OBJECTIVES**

Our objective is to evaluate and refine the techniques needed to test the hypothesis that brief exposure to high turbulence levels (such as those occurring during episodic wind events) will break long chains of fragile diatom species and damage or even kill individual cells of sensitive species thus altering the particle characteristics, size structure, and composition of the phytoplankton community and the resulting bulk optical properties of the water column. We are particularly interested in testing and refining the ability of our CytoSense submersible scanning flow cytometer to quantify *in situ* not only the abundance, length and bio-optical characteristics of large non-spheroid phytoplankton, but also the occurrence of broken chains and damaged or dead cells. We are also interested in testing our lab-based models that predict that growth, chain length and morphology of large chain forming diatoms are affected by sustained exposure to low and moderate levels of turbulence during bloom development.

### **APPROACH**

Our approach during this grant was to conduct field experiments designed to test the *in situ* scanning flow cytometry and bio-optical profiling techniques needed to quantify the effects of episodic enhanced turbulent events on large non-spheroid phytoplankton and the bulk inherent optical

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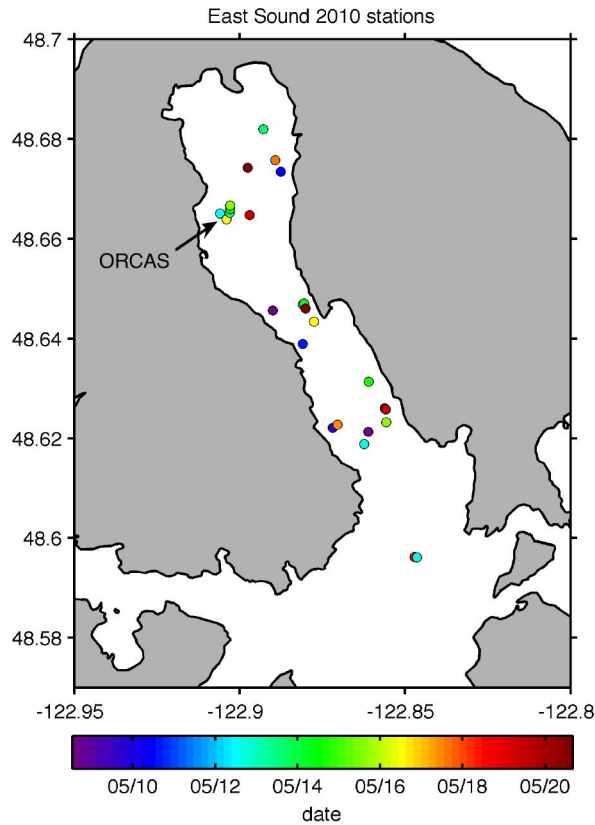
properties of the water column. Our approach had four components. First, we conducted these experiments in East Sound, WA, (a 2 by 12 km by 30 m deep fjord) where topography constrains lateral advection thus allowing the autonomous moored profilers and small boats to repeatedly sample the persistent thin layers of non-spheroid phytoplankton that frequently develop in this system between turbulent mixing events. Second, we used an ORCAS autonomous moored profiler to collect the time series data needed to detect turbulent mixing events and the changes in fine-scale optical, chemical and physical structure during and after the event. Third, we used our surface-deployed high-resolution profiler to measure temporal and spatial variation in fine-scale optical structure of dissolved and particulate material inside East Sound and in adjacent waters. Fourth, we used the data from our high resolution profiler to select locations for collection of phytoplankton samples from features of interest for immediate on-board analysis of individual particle characteristics using video microscopy and scanning flow cytometry.

## **WORK COMPLETED**

We have completed the processing and initial analysis of data from the 2009 experiment. We have completed counting of the discrete phytoplankton samples collected in the 2009 cruise and used the resulting data to verify our CytoSense based estimates of changes in the abundance of live and dead cells of the dominant species. We have presented the results of the 2009 cruise at the 2010 Ocean Sciences meeting. We conducted our second cruise during May 2010. During this cruise we tested the three sampling techniques as described below.

*Autonomous profiler measurement of temporal variation in fine-scale physical and optical structure:* We deployed one of our ORCAS autonomous moored profilers equipped with a Nortek Vector velocimeter in western upper East Sound (arrow, Figure 1). The ORCAS profiler measured temporal changes in the fine-scale vertical structure of (a) spectral absorption and attenuation by dissolved plus particulate material, (b) optical backscatter, (c) chlorophyll-a fluorescence, (d) fluorescence of colored dissolved organic matter, (e) temperature, (f) salinity, (g) density, (h) oxygen, and (i) small-scale currents, current shear, and turbulence. These profiles were collected every other hour.

*High-resolution profiler measurement of fine-scale physical and optical structure:* We used our slow-descent high-resolution profiler to collect 66 profiles of fine-scale vertical structure of (a) spectral absorption and attenuation by dissolved plus particulate material at 9 wavelengths (b) spectral absorption and attenuation by dissolved plus particulate material at 86 wavelengths, (c) spectral absorption of colored dissolved organic material at 9 wavelengths using a WET Labs ac-9 with 0.2 micron pre-filter, (d) optical backscatter at 3 angles, (e) spectral optical backscatter at 3 wavelengths and at 9 wavelengths, (g) chlorophyll-a fluorescence, (h) fluorescence of colored dissolved organic matter, (i) temperature, (j) salinity, (k) density, and (l) oxygen. The addition of WET Labs ac-s absorption and attenuation meter and a WET Labs BB9 backscattering sensor to our high-resolution profiler this year increased the spectral resolution of our absorption and scattering measurements while at the same time providing replicate measurements at wavelengths where the sensors overlapped. The resulting data were used to calculate the vertical fine-scale structure of absorption and scattering by particulate material and a series of derived parameters used to further optically characterize the particulate and dissolved material. Replicate profiles were collected each day at a series of locations inside East Sound and in adjacent waters (Figure 1).



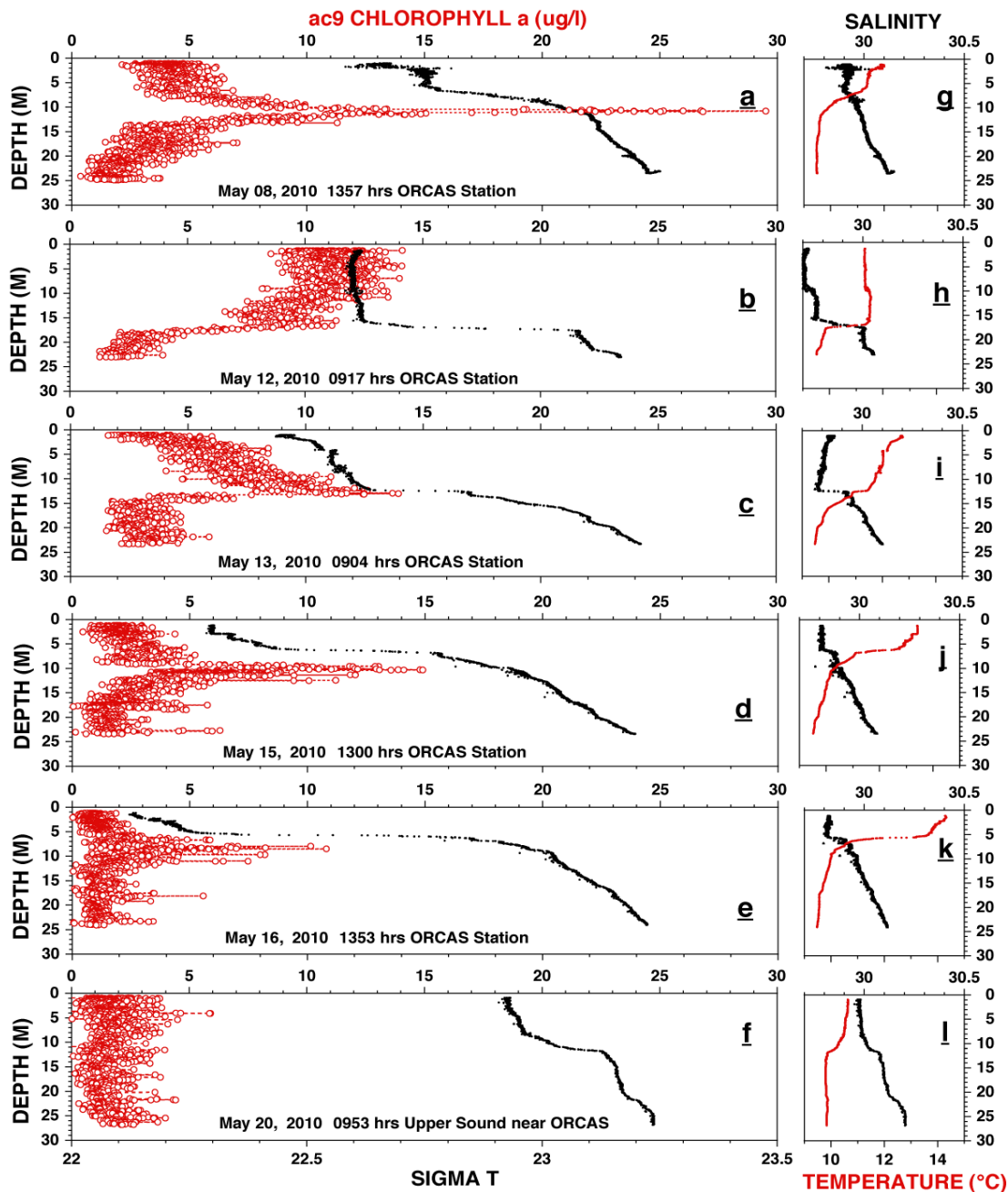
**Figure 1: Location of 2010 profiles in East Sound and adjacent waters of fine-scale vertical physical and bio-optical structure collected using the ORCAS autonomous moored profiler (arrow pointing to center of cluster) and the high-resolution profiler (colored circles). Samples for individual particle analysis using microscopy and CytoSense scanning flow cytometry were collected at one or more depths at most of the high-resolution profiler stations.**

*On-board analysis of individual particle characteristics:* We used our small boat to collect 67 discrete samples from features of interest for on-board comparative analysis of individual particle characteristics using video microscopy and CytoSense scanning flow cytometry. Sub-samples were preserved for later analysis in the lab of phytoplankton composition and abundance. We used the video microscope to characterize (a) the composition, size and morphology of the large phytoplankton in each sample, and (b) the presence of dead cells and broken chains. We used CytoSense to measure the side scattering, forward scattering and spectral fluorescence (5 wavelengths) at 0.5 micron intervals along the length of each particle. Comparisons with the microscope data were then used to evaluate the ability of CytoSense to discriminate and enumerate particle types, as well as determine their length, viability and physiological characteristics.

## RESULTS

*Evolution of fine-scale physical and optical structure in East Sound during May 2010.*

2010 was characterized by the presence of a very complex phytoplankton community that developed under variable weather conditions that included sunny periods that warmed surface waters and encouraged layer development, followed by short wind events that reduced stratification and dispersed thin layers (Figure 2). For example, south wind events on May 10 and 11 dramatically reduced vertical



**Figure 2.** Temporal changes in upper western East Sound between May 8 and May 20, 2010 in fine-scale vertical structure of phytoplankton biomass (chlorophyll-a calculated from ac-9 data, red circles) and density (Sigma T, black dots) are shown in Figure 2 a-f, and the corresponding changes in the vertical structure of temperature (red dots) and salinity (black dots) in Figure 2 g-l. This figure shows that while phytoplankton vertical structure was initially dominated by a thin layer on May 8 (a), it became a thick surface layer immediately following the south wind event on May 11 (b) that moved deeper in the water column to form a chlorophyll maximum at mid-depths (c-e). This chlorophyll-a maximum declined in intensity over time (d, e) until it was completely eliminated during the wind event on May 19 (f). The figure also shows a steady warming of the surface layer during the calm, sunny weather that occurred between wind events on May 11 and May 19.

stratification down to 18 m in upper East Sound (compare Figures 2 g and 2 h) and replaced the intense thin layer observed on May 8 (Figure 2a) with a broad chlorophyll maximum in surface waters (Figure 2b). Mostly sunny weather over the next few days warmed surface waters (Figure 2 i-k) thus providing conditions favorable for decline of phytoplankton abundance in surface waters and the formation of a mid-depth chlorophyll maximum (Figure 2 c-e). This chlorophyll maximum declined in intensity over time (Figure 2 d-e) until it was completely eliminated during the wind event on May 19 (Figure 2 f). Although thin layers were periodically seen elsewhere in East Sound during this sunny period, they never reached the intensity of the thin layer seen on May 8-9 in the upper sound.

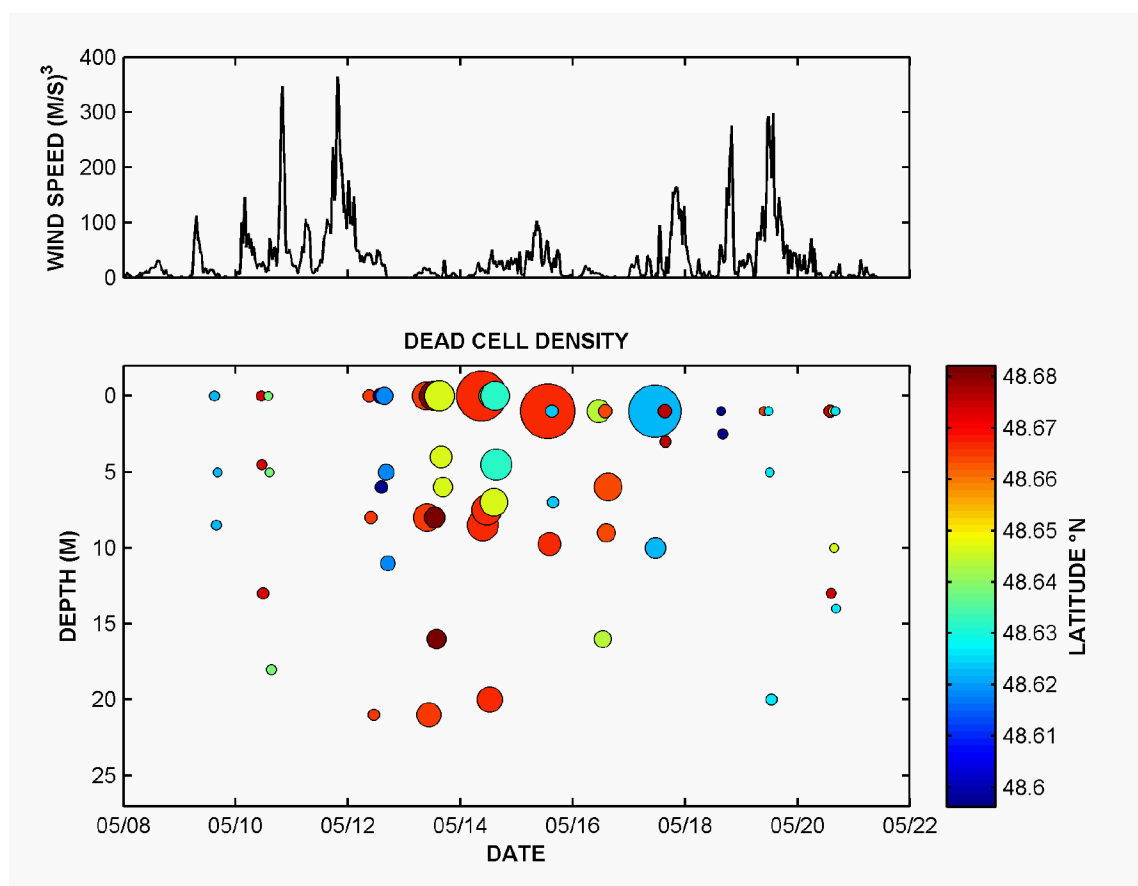
*Evolution of phytoplankton community structure and individual particle characteristics:* In contrast to the monospecific *Haslea* bloom of 2009, the phytoplankton community during our 2010 experiment was incredibly diverse and dominated by chain-forming diatoms (Figure 3). The genus *Chaetoceros* was especially well represented, with > 20 species. Many of these chain-forming diatoms formed large colonies that ranged up to a millimeter in length. These colonies varied in shape from long and linear, to spirals and large globular colonies (Figure 3). Although less abundant, the assemblage included



**Figure 3. Representative healthy diatoms from early in the experiment, including *Chaetoceros vanheurckii*, *Chaetoceros debilis*, *Chaetoceros socialis*, *Chaetoceros laciniosus*, *Thalassionema nitzschioides* and *Asterionellopsis glacialis*.**

many additional diatoms and several dinoflagellates, including the heterotrophs *Noctiluca scintillans* and *Protoperdinium* spp. Visual (microscopic) examination of the diatoms suggested that they became less healthy as the experiment progressed. Over time, phytoplankton diversity continued to decline and large, flocculent aggregates formed. CytoSense measurements of the abundance of live and dead cells indicated that while there were very few dead cells in the water column prior to the May 11 wind event, the abundance of dead cells increased following the storm, reaching a peak that occurred several days after the storm (Figure 4). This peak in the abundance of dead cells coincided with the decline in



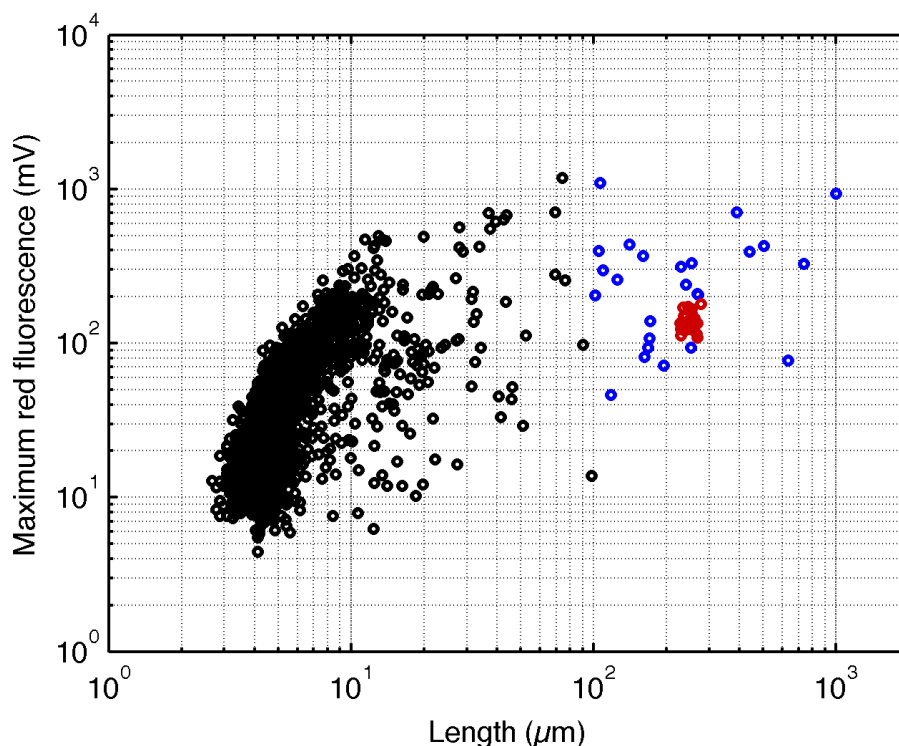


**Figure 4. Variation in cubed wind speed and CytoSense based estimates of temporal changes in the abundance of dead cells in East Sound between May 8 and 20, 2010. The abundance of dead cells is indicated by the size of the circle and the location along the axis of the sound is indicated by the color code at the right of the figure (with red being upper and blue being lower East Sound). The figure shows that the number of dead cells increased from very low numbers to a peak several days after the storm on May 11.**

both phytoplankton biomass observed in the chlorophyll data (Figure 2 b-d) and diversity seen in the microscopy data. This clearly indicates that cell mortality played a key role in the declines in abundance and diversity of large phytoplankton following the wind mixing events on May 10 and 11. Microscopy observations on May 17 and 18 indicated that some large species such as *Chaetoceros socialis* were still quite abundant. After a sudden, intense storm on May 19, the water column cleared in upper East Sound leaving chlorophyll levels exceedingly low (Figure 2f). This event reset the system, and set the stage for the next successional bloom. At a surface station in upper East Sound, microscopy revealed that the once-abundant *Chaetoceros* were gone and the community was now dominated by three needle-like genera (Figure 5), which had constituted part of the ‘hidden flora’ two weeks earlier: *Pseudo-nitzschia*, *Rhizosolenia* and *Haslea*. All of these species are less than 10 microns wide but have chains or individual cells or chains that exceed 100  $\mu\text{m}$  in length (Figure 5). CytoSense analysis of these samples documented a variety of phytoplankton particles > 100  $\mu\text{m}$  in length and with a maximum red fluorescence (chlorophyll) > 30 mV (Figures 5 and 6). Although we were able to use comparisons with our 2009 pulse profile data to rapidly identify some of these large particles as *Haslea* (Figure 7a), the diversity of the community made it difficult to match the remaining observed individual pulse profiles (Figure 7 b-e) to specific phytoplankton species.

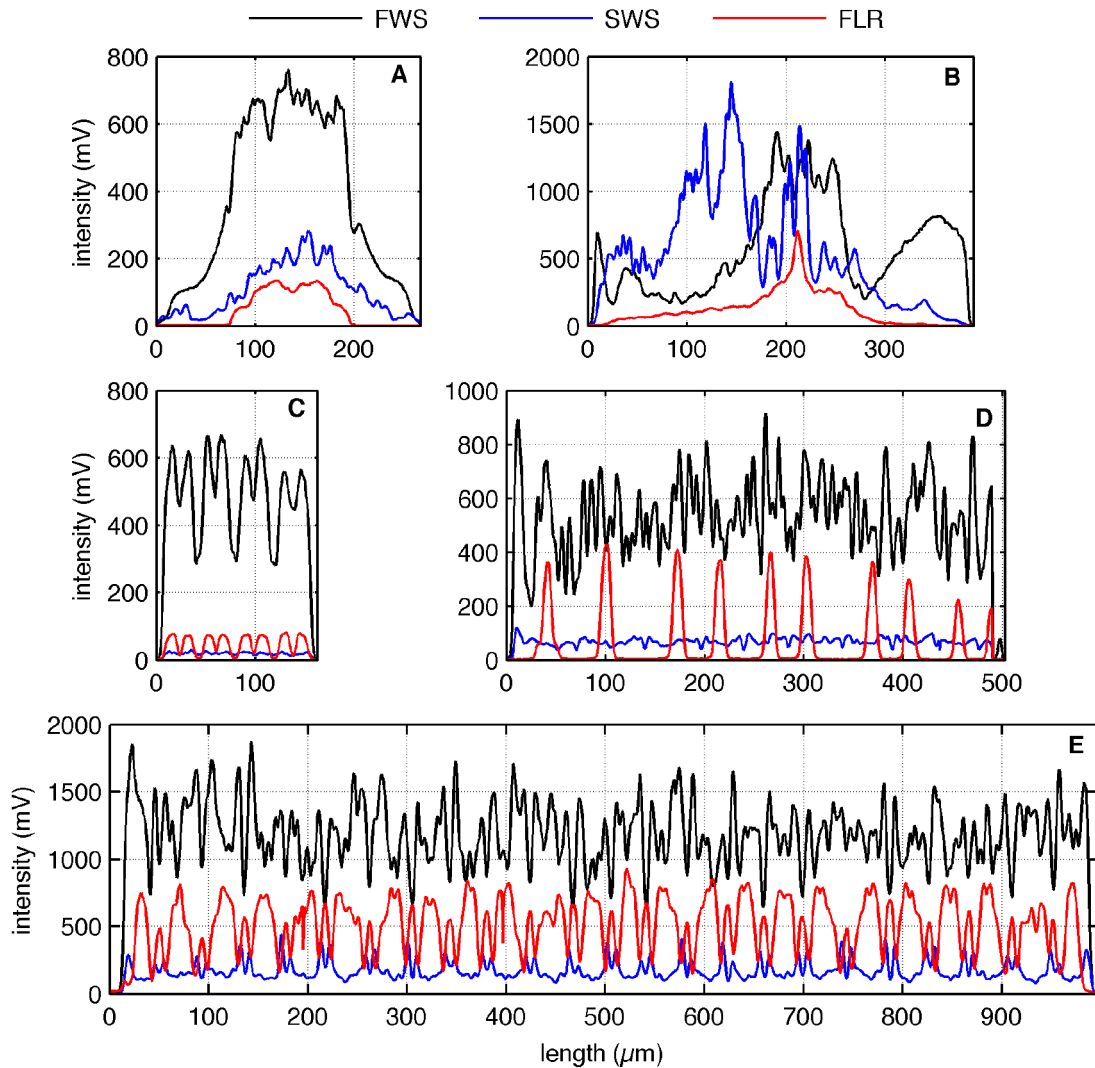


**Figure 5.** Representative healthy diatoms from the surface layer in upper East Sound following the storm on May 19, 2010. The figure shows that needle-shaped diatoms now predominate, including *Rhizosolenia pungens*, *Pseudo-nitzschia* spp., and *Haslea* cf. *wawriake*.



**Figure 6.** CytoSense cluster plot of red chlorophyll-a fluorescence versus particle length for a 1 ml sample collected after the storm from the surface mixed layer in upper East Sound. The large phytoplankton (defined as chlorophyll-a containing particles of length  $> 100\mu\text{m}$  and red fluorescence (chlorophyll)  $> 20\text{ mV}$ ) are depicted in color with the pennate diatom *Haslea* cf. *wawriake* denoted by red circles and the other species denoted by blue circles. The figure shows that there was no evidence of dead *Haslea* or the other large phytoplankton following the May 19 storm.





**Figure 7.** CytoSense pulse profiles of forward scatter (FWS) (black lines), side scatter (SWS) (blue lines) and chlorophyll-a red fluorescence (FLR) (red lines) for representatives of the large non-spheroid particles highlighted in color in Figure 6. The figure illustrates that while some particles differed dramatically both in the relative magnitude and along-particle variation in forward and side scatter (compare 7 a, b and c), other particles had a very similar scattering pulse shapes, but varied significantly in the along-axis continuity and magnitude of red fluorescence (compare 7 d, e). *Haslea cf. wawriake* (7 a) was easy to identify based on comparison to our 2009 data. All others have complex signatures, which cannot be assigned with certainty to the taxa present in our samples. Based on comparison with our live microscopy images, (B) might represent a partial *Chaetoceros* colony, and (C) might be a short chain of *Pseudo-nitzschia*. It is difficult to determine if (D) and (E) are different taxa, or the same species in different physiological condition. We can hypothesize, but not be certain, that they might represent *Leptocylindrus minimus* or *Guinardia delicatula*. Addition of the Image-in-flow module to our CytoSense will ameliorate these problems by giving us the ability to use the pulse profiles to trigger photomicrographs of the phytoplankton.

## IMPACT/APPLICATION

This project has several important impacts/applications. First, the excellent agreement in both years between video microscopy and flow cytometry measurements of individual particle characteristics clearly demonstrates that CytoSense scanning flow cytometry can be used in the field for *in situ* quantification of the effects of environmental conditions on the particle length, abundance and viability status of large non-spheroid phytoplankton in natural plankton assemblages. This is a huge breakthrough given the extreme difficulty in measuring *in situ* changes in the abundance and mortality of individual phytoplankton species using other methods. This initial success opens the door to deploying CytoSense on our ORCAS profilers so that it can be used *in situ* to quantify changes in particle length and viability under storm conditions that prohibit surface vessel operations. Second, the strong correlation between the increased abundances of dead large phytoplankton detected by CytoSense following the storm and the decrease phytoplankton biomass evident in the optical profiler data and decrease in diversity seen in the microscopy data provides strong support for the hypothesis that mortality played a key role in the decline in the phytoplankton diversity and biomass following the storm. This clearly demonstrates how *in situ* measurements by CytoSense can be used to increase our understanding of the mechanisms controlling bloom dynamics and temporal changes in optical structure. Third, this cruise has provided us with a field data set that will allow us to evaluate the extent to which measurements of optical properties of individual particles (and their sub-particle optical structure) can be used to help us interpret the inherent optical properties measured by our IOP sensors as well as the optical structures measured by airborne lidar. Such analyses will allow us to evaluate CytoSense as a tool for improving optical inversion models as well as for studying phytoplankton ecology and patch dynamics. We view this and subsequent field tests as a critical step in helping this new technology to realize its potential to revolutionize interdisciplinary oceanographic studies of phytoplankton ecology by creating a bridge between characterization of the phytoplankton community using microscopy and fine-scale optical structure measured using *in situ* and remote sensing techniques.

## RELATED PROJECTS

This project has been coordinated with our lidar validation project entitled "*In situ* Validation of the Source of Thin Layers Detected by NOAA Airborne Fish Lidar". This lidar project was funded under ONR grant number N000140410276 to Percy L. Donaghay (PI), Jan Rines and James Sullivan at URI and a companion ONR contract number N00014091P20039 to James Churnside (PI) at NOAA. This project has also been coordinated with our NOPP holocamera project entitled "A submersible holographic camera for the undisturbed characterization of optically relevant particles in water (HOLOCAM)". This project was funded with James Sullivan at WET Labs as the lead PI, with subcontracts to Percy Donaghay (PI, URI subcontract) and Joseph Katz (PI, JHU subcontract).